NOTE TO THE FILE
BNF0066
April 4, 2000

Subject: Aventis's (formerly AgrEvo USA Company) Male Sterile Corn, *MS6* line.

Keywords:
Corn, SeedLink™ corn, *Zea mays*, *Barnase* gene, Ribonuclease (RNase), Male Sterility, *bar* gene, Phosphinothricin acetyltransferase gene, PAT gene, Herbicide- Tolerant, Glufosinate-Tolerant, *Bacillus amyloliquefaciens*.

Background:

In a submission dated June 7, 1999, Aventis (formerly AgrEvo USA Company) provided summary information to support the safety assessment of their "Male Sterile" corn, MS6 line (also known as SeedLink™ corn). In a submission dated February 7, 2000, Aventis provided additional information regarding the level of some amino acids in their "Male Sterile" corn.

Intended effect and food/feed use

The intended effect of the MS6 genetic modification is to produce a male sterile corn line to provide a more reliable pollination control system for hybrid seed production in corn. The transgenic MS6 corn developed by Aventis, contains the *barnase* gene from the bacterium *Bacillus amyloliquefaciens*. The *barnase* gene encodes a ribonuclease which catalyzes the hydrolysis of single stranded RNA molecules and is expressed in the anther only. The anthers of the MS6 corn become incapable of producing viable pollen grains. The *barnase* gene is linked to the glufosinate tolerance gene (*bar* gene from *Streptomyces hygroscopicus*) which was used to select the transformed cells. According to Aventis, the two genes will co-segregate as a single locus to maintain the male sterile line through crossing with wild type plants followed by the application of the herbicide for efficient rouging of fertile plants in a segregating population for quality assurance in the hybrid seed production.

Molecular alterations and characterization

Aventis transformed the tissue derived from corn H99 inbred line using the microprojectile bombardment technique to produce the transformation event MS6 corn. The transformation vector pEV136 consists of P35S-*bar*-3' nos and PCA55-*barnase*-3' nos gene constructs. The chimeric gene P35S-*bar*-3' nos contains the constitutive 35S promoter of the cauliflower mosaic virus (P35S), the *bar* coding sequence conferring tolerance to the herbicide glufosinate ammonium, and the 3'-untranslated region of the nopaline synthase gene from the T-DNA of pTiT37 (3' nos). The PCA55-*barnase*-3' nos gene construct contains a promoter fragment from a *Zea mays* anther-specific gene (PCA55), the coding region of the ribonuclease gene of *Bacillus amyloliquefaciens* (*barnase*), and the 3'-untranslated region of the nopaline synthase gene from the T-DNA of pTiT37 (3'nos). The pEV136 plasmid also contains an origin of replication (*ori*) required for replication of the plasmid in *E.coli*, and the *B-lactamase* gene

(*bla*) which confers resistance to the antibiotic ampicillin to ensure retention of the plasmid in the bacterial host.

According to Aventis, maintenance and multiplication of the male sterile line was accomplished by crossing the male sterile plant (as a female parent) with a fertile plant. Since the *barnase* and *bar* genes are physically linked, the genes segregated in the genetic offspring as a single locus in a 1:1 segregation ratio (herbicide tolerant and male sterile: herbicide susceptible and male fertile). Aventis eliminated the latter phenotype in each generation of a multiplication or backcrossing program by treating the plants with glufosinate-ammonium. The results of field experiments confirmed that Mendelian inheritance is normal with no evidence of genetic instability of the *barnase* gene.

To determine the nature and number of *barnase*, *bar* and *bla* gene insertions in the transformation event MS6, the notifier conducted Southern hybridization of genomic DNA from event MS6 and from non-transgenic H99 inbred lines. Data showed that the MS6 corn contains only small parts of the *ori* and *bla* sequences, one copy of *barnase*, and two copies of the *bar* gene and that the genetic insertion was at a single locus site in the corn genome.

Aventis performed Southern analysis of genomic DNA of transgenic plants from multiple generations and different genetic backgrounds using the PCA55 probe. The banding pattern ("fingerprint") was identical across generations and genotypes confirming the genetic stability of event MS6.

Expression of genes in transformation event MS6

The notifier conducted an enzyme linked immunosorbent assay (ELISA) to measure the level of expression of the phosphinothricin acetyltransferase (PAT) protein (a product of the *bar* gene) in forage, fodder, and grains of the MS6 transgenic plants that were treated with Liberty® herbicide to destroy any glufosinate susceptible plants. According to Aventis, the PAT protein was expressed at very low levels in these products, and was not detected in oil obtained from dry milled grains or in any of the wet milled products tested by Aventis.

Expression of the *barnase* gene in any cell other than tapetal cell layer of anther would lead to disruption of normal cell function and result in abnormal plant growth. The notifier noted that, with the exception of the male sterility trait, the transgenic plants containing event MS6 developed in a comparable manner to non-transgenic plants. Aventis concluded that the expression of the *barnase* gene is limited to the tapetal cell layer.

Aventis performed Northern analysis of RNA extracted from leaf, root, and seed from the transgenic corn containing the event MS6 and confirmed that the *bla* gene fragment present in event MS6 is not expressed in the transgenic plants.

Compsitional profile

Corn grain is fed primarily to animal livestock, with very little corn used for direct human consumption. Processed corn grain (oil or starch) is used for food and numerous industrial applications.

The SeedLink™ corn comprises two linked components: the male sterility and the glufosinate tolerance. The male sterility component is to provide a more reliable pollination control system for hybrid seed production in corn, whereas the glufosinate tolerant component is for efficient field selection of transformed plants. Aventis did not anticipate any other effect from the introduction of the *barnase* and *bar* genes into corn. Nonetheless, Aventis conducted extensive compositional analyses of forage (silage) and grains from the non-transgenic and the transgenic MS6 corn lines to confirm that there were no unintended effects of the genetic modification.

Grains:

Aventis determined the composition profile of grains from transgenic MS6 and from the non-transgenic corn plants. According to the notifier, the level of proximates (moisture, ash, crude fat/ oil, crude fiber, acid detergent fiber, carbohydrates) and minerals (calcium and phosphorus), as well as the amino acid profile of the transgenic MS6 corn hybrid were similar to that of the non-transgenic corn and to the reported literature values for corn. Compared to the literature values, the content of crude protein was low in both transgenic and nontransgenic corn, whereas the level of neutral detergent fiber was slightly higher in transgenic corn. AgEvo stated that these differences could be related to the early harvest of the tested plants.

Corn oil is popular as a food oil in part because of its high content of polyunsaturated fatty acids (linoleic and linolenic). Aventis also measured the level of fatty acids in transgenic MS6 and non-transgenic corn and concluded that the fatty acid profile of the transgenic is similar to the non-transgenic corn and is consistent with the published USDA values.

Silage:

One of the major uses of corn is as silage for animal feed. Silage is defined as the green tissue harvested at the late milk or early dough stage of the plant development. Aventis analyzed the nutritional composition of silage from transgenic MS6 corn hybrid plants and from the non-transgenic corn hybrid plants. The composition profile included: moisture, crude fat/ oil, protein, ash, acid detergent fiber, neutral detergent fiber, carbohydrates, calcium, and phosphorus. According to the notifier, the level of proximates of the transgenic MS6 corn hybrid was similar to that of the non-transgenic corn and to the reported literature values for corn. In the notice, Aventis stated that the content of crude fat/ oil was low in both transgenic and nontransgenic corn as compared to the literature values, and that this could be due to the maturity level at harvest (plants were harvested at a physiologically early stage of development).

Conclusions

Aventis has concluded that its transgenic male sterile corn hybrid containing transformation event MS6 is not materially different in terms of food safety and nutritional profile from non- transgenic corn hybrids currently on the market. At this time, based on Aventis's description of its data and analysis, the agency considers Aventis's consultation on MS6 corn line to be complete.

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